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Factors Affecting Germination of Oospores of *Phytophthora infestans*

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With 4 figures

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Abstract

When oospores from the pairing between A^1 and A^2 mating types of *Phytophthora infestans* were treated with 0.25 % $KMnO_4$ solution for 15 min and incubated at 19 °C under light on a modified S+L medium, germination commenced within 4 days and reached about 70 % after 20 days. Under these conditions, more than 25 % of oospores obtained from a 4-day-old culture germinated. To obtain a high germination rate of *P. infestans* oospores, light was essential during germination but not during growth and oospore formation. The optimum time for activation of oospores with 0.25 % $KMnO_4$ was 15 to 30 min and a suitable concentration of $KMnO_4$ for 15 min activation was 0.25 to 0.45 %.

Zusammenfassung

Faktoren, die die Keimung von *Phytophthora infestans*-Oosporen beeinflussen

Wenn Oosporen aus der Paarung zwischen den A^1- und A^2-Paarungstypen von *Phytophthora infestans* mit einer 0,25%igen $KMnO_4$-Lösung 15 Minuten lang behandelt worden waren und anschließend bei 19 °C unter Licht auf einem modifizierten S+L-Nährmedium inkubiert wurden, fing die Keimung innerhalb von 4 Tagen an, und nach 20 Tagen waren circa 70 % der Oosporen ausgekeimt. Unter diesen Bedingungen keimten mehr als 25 % der von 4 Tagen alten Kulturen gewonnenen Oosporen aus. Licht war für eine hohe Keimungsrate der *P. infestans*-Oosporen essentiell, aber nicht während des Wachstums bzw. der Oosporenbildung. Die optimale Einwirkungszeit der 0,25%igen $KMnO_4$-Lösung bei der Oosporenaktivierung lag zwischen 15 und 30 Minuten, und bei einer Aktivierungszeit von 15 Minuten lag die brauchbare $KMnO_4$-Konzentration zwischen 0,25 und 0,45 %.

Inability to obtain a consistent and high germination rate of oospores is one of the main obstacles in obtaining reliable data for genetic studies of *Phytophthora* (RIBEIRO 1978, ZENTMYER and ERWIN 1970). Oospores produced by self-inducing (homothallic) species of *Phytophthora* are in general easier to germinate than those produced by cross-inducing (heterothallic) species (ZENTMYER and ERWIN 1970). A high germination rate of oospores has been reported for self-inducing species of *P. cactorum* (Lebert & Cohn) Schröter (BANIHASHEMI and MITCHELL 1976), *P. heveae* Thompson (LEAL and GOMEZ-MIRANDA 1965), *P. katsurae* Ko & Chang (KO and ARAKAWA 1980), *P. syringae* Kleb. (HARRIS and COLE 1982), *P. criticola* Sawada (PLOURDE and GREEN 1982), *P. fragariae* Hickman (DUNCAN 1985), *P. vignae* Purss (ISHIGURO and UI 1981), and *P. megasperma* Drechsler (EL-HAMALAWI and ERWIN 1986). An inconsistent and low rate of germination of oospores has been reported for cross-inducing species of *P. palmivora* (Butler) Butler (KAOSIRI *et al.* 1980), *P. capsici* Leonian (RIBEIRO *et al.* 1975), *P. parasitica* Dastur (BOCCAS 1981), *P. cambivora* (Petri) Buisman (RIBEIRO *et al.* 1975), and *P. infestans* (Mont.) de Bary (SHATTOCK *et al.* 1986a, 1986b).

Recently a method for inducing a consistent high rate of oospore germination of *P. parasitica* has been developed (ANN and KO 1988). The method consists of exposing oospores to light during maturation and treating oospores with 0.25 % $KMnO_4$ for 20 min before incubation under light on an agar medium containing basal salts, glucose and lecithin. When this method was used to induce oospore germination of *P. infestans*, the germination rate was increased only slightly in comparison with the control. A consistent high germination rate of oospores was achieved when the method of Ann and Ko was modified using information derived from the study of factors affecting oospore germination of *P. infestans*. Details of the study are reported here.

Materials and Methods

Microorganisms

Isolates 533 (A^1) and 550 (A^2) of *P. infestans* were obtained from Dr. W. E. Fry. The former was from a commercial potato field at El Tecolote, Mexico and the latter was from a wild *Solanum* species at Tenango, Mexico (TOOLEY *et al.* 1985). The isolates which have originated from single zoospores were maintained on V-8 rye agar modified from the rye agar described by CATEN and JINKS (1968). Rye broth was obtained by soaking 50 g whole rye grains in 1100 ml distilled water at 24 °C for 24—36 h followed by autoclaving at 120 °C for 30 min, filtration through four layers of cheesecloth and adjusting the final volume to 1000 ml with distilled water. V-8 rye agar was prepared by adding 5 % V-8 juice, 0.02 % $CaCO_3$ and 2 % Bacto agar to rye broth.

Formation of oospores

Two pieces of agar cultures (ca. 3 × 3 × 3 mm) of the opposite mating type were placed 5 mm apart on a V-8 rye agar block (ca. 15 × 15 × 3 mm) in the centre of a small Petri dish (60 mm). These dishes were then sealed with 2 layers of parafilm and incubated in darkness for 20 days at 19 °C for formation and maturation of oospores unless otherwise stated.

Germination of oospores

Oospore suspensions were obtained by triturating each culture block (ca. 15 × 15 × 3 mm) containing oospores with 50 ml of distilled water in an Omni mixer at 4,500 rpm for 1 min. The suspension was filtered successively through a 53-μm and a 20-μm sieve. Oospores retained on the 20-μm sieve were washed with tap water and resuspended in 10 ml of sterile distilled water. The oospore suspension was mixed with an equal volume of freshly prepared $KMnO_4$ solution at 0.5 % (w/v). After agitating the mixture for 15 min on a shaker, oospores were washed free of $KMnO_4$ on a 20-μm sieve with tap water. About 100—200 oospores were spread on S+L medium of RUBEN *et al.* (1980) amended with 0.01 % asparagine and 2 % Bacto agar. After autoclaving, this medium had been supplemented with 100 μg of ampicillin, 50 μg of nystatin and 10 μg of pentachloronitrobenzene per millilitre to prevent growth of possible contaminants. After incubation at 19 °C under continuous cool white fluorescent light (2,000 lux) for 20 days, the germination rate was assessed microscopically.

Three replicates were used for each treatment and all the experiments were repeated at least twice.

Results

To determine the time required for activation of oospores with $KMnO_4$, oospores of *P. infestans* were agitated in 0.25 % $KMnO_4$ solution for 0, 5, 15, 30, 50 and 75 min before being washed and plated on modified S+L medium. Germination of oospores increased with increasing time of treatment with 0.25 % $KMnO_4$ solution up to 15 min. The optimum time for activation with $KMnO_4$ was 15 to 30 min. After 30 min, germination decreased as time of treatment increased (Fig. 1). The difference between percentages of germination of oospores treated for 15 and 30 min was not statistically significant at the 5 % level. However, these germination rates were significantly higher than that treated for 5 or 50 min.

Oospores were treated for 15 min with $KMnO_4$ at concentrations ranging from 0.125 to 2 % to determine the concentration of $KMnO_4$ required for activation of oospores of *P. infestans*. Germination of oospores increased with increasing concentration of $KMnO_4$ up to 0.25 %. Of the treatments used the best concentration of $KMnO_4$ for activation of oospores was within the range of 0.25 to 0.45 %. Germination decreased when the concentration of $KMnO_4$ was

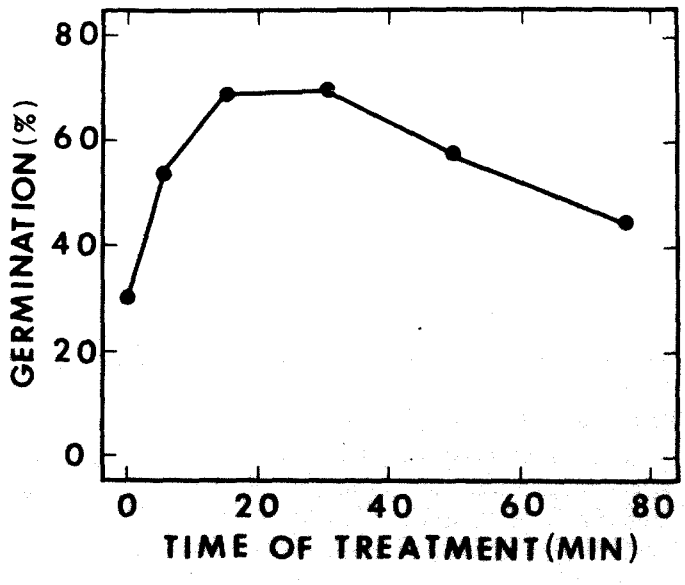

Fig. 1. Germination of oospores from pairing of A^1 (533) and A^2 (550) mating types of *P. infestans* after treatment with 0.25 % $KMnO_4$ solution for 0, 5, 15, 30, 50 and 75 min. Germination was recorded after incubation on modified S+L medium at 19 °C under light for 20 days

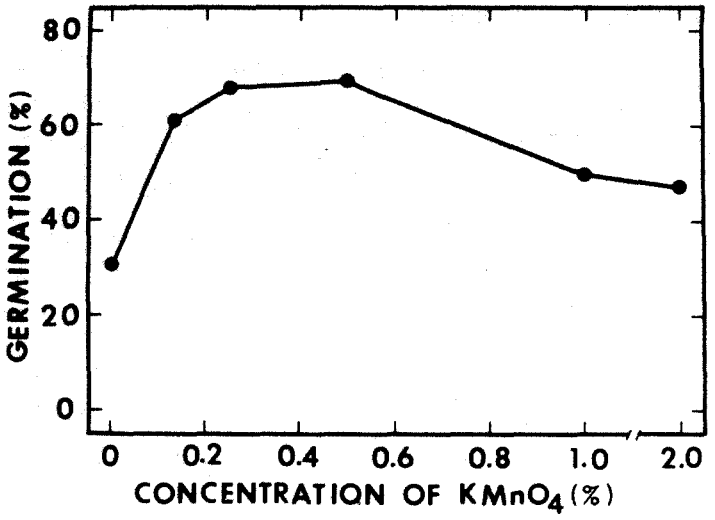

Fig. 2. Germination of oospores from pairing of A^1 (533) and A^2 (550) mating types of *P. infestans* after treatment for 15 min with various concentrations of $KMnO_4$ solution. Germination was recorded after incubation on modified S+L medium at 19 °C under light for 20 days

increased to 1 or 2 % (Fig. 2). The difference between percentages of germination of oospores treated with $KMnO_4$ at the concentrations of 0.25 and 0.45 % was not statistically significant at the 5 % level. However, these germination rates were significantly higher than that for those treated with 0.125, 0.1 or 0.2 % $KMnO_4$. Activation of oospores with $KMnO_4$ at the concentration of 0.25 % for 15 min was selected for subsequent experiments. Under these conditions, oospore germination of *P. infestans* commenced within 4 days, reached 40 % in 5 days and was as high as 70 % after 20 days (Fig. 3).

The effect of light during growth and oospore formation on oospore germination was studied by incubating paired cultures of *P. infestans* under light or in darkness at 19 °C for various periods of time before processing and activation of oospores. These periods of time are designated the age of culture in this study. After activation with $KMnO_4$, oospores from each treatment were germinated on modified S+L medium under light or in darkness at 19 °C to determine the effect of light during germination on germination rate. Germination was recorded after incubation for 20 days. Results show that to obtain a high

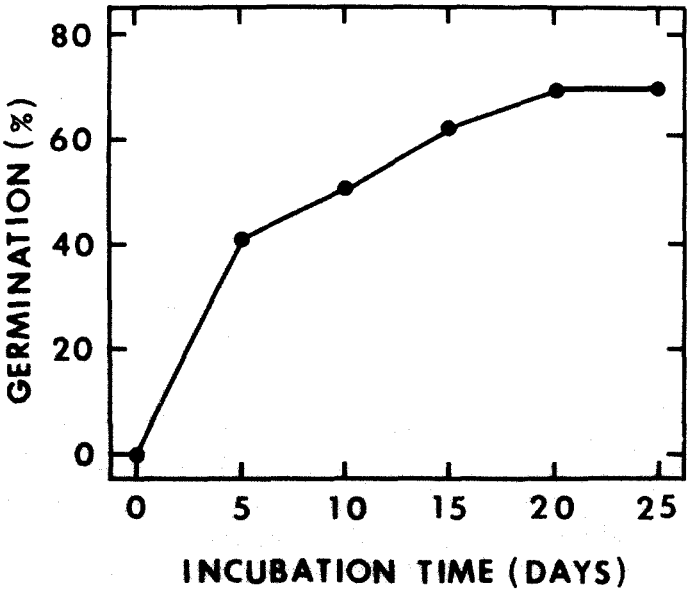

Fig. 3. Germination of oospores from pairing of A^1 (533) and A^2 (550) mating types of *P. infestans* after treatment with 0.25 % $KMnO_4$ solution for 15 min. Germination was recorded after incubation on modified S+L medium at 19 °C under light for 5, 10, 15, 20 and 25 days

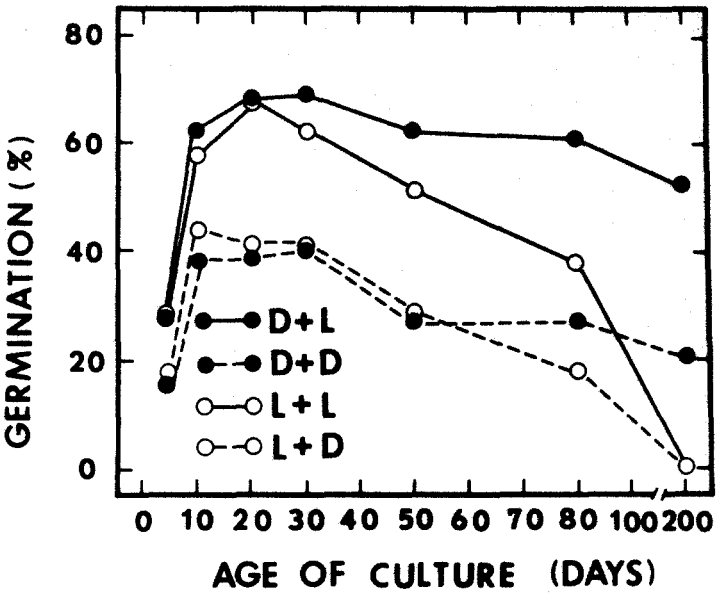

Fig. 4. Effect of age and light on germination of oospores from the pairing of A^1 (533) and A^2 (550) mating types of *P. infestans*. Oospores were treated with 0.25 % $KMnO_4$ for 15 min followed by incubation on modified S+L medium at 19 °C for 20 days. L+L, oospores exposed to light during formation and during germination; L+D oospores exposed to light during formation but kept in darkness during germination; D+L, oospores kept in darkness during formation but exposed to light during germination; D+D, oospores kept in darkness during formation and during germination

germination rate at a culture age of 80 days or younger exposure to light during germination is more important than during growth and oospore formation (Fig. 4). For instance when oospores were obtained from 20-day-old cultures, the germination rate was about 70 % if oospores were exposed to light during germination. The difference in germination rates between those cultured under light and in darkness was not statistically significant. The germination rate was about 40 % if oospores were kept in darkness during germination, and the difference in germination rates between those cultured under light and in darkness was also not statistically significant. When *P. infestans* was exposed to light during germination, more than 25 % of those oospores obtained from 4-day-old cultures were able to germinate and germination of oospores from 10-day-old cultures was about 60 %.

When *P. infestans* was cultured under light, some oospores germinated on agar medium and the number of germinating oospores increased with increasing age of culture. Exposure of the culture to light had an adverse effect on oospores germination when the fungus became relatively old. For example, at the culture age of 80 days and with light during germination, 61 % of the oospores from the culture grown in darkness germinated, while of those from the culture grown under light 38 % germinated (Fig. 4). When germination was carried out in darkness, 27 % of the oospores from cultures grown in darkness germinated, while only 18 % of those from cultures grown under light germinated. The difference was statistically significant at the 5 % level in both cases.

Discussion

Previous methods for inducing oospore germination of *P. infestans* included treatment with horse dung infusion (SMOOT *et al.* 1958), cold treatment at 4 °C (ROMERO and ERWIN 1969) and ingestion by water snail (SHATTOCK *et al.* 1986a, 1986b). Athough germination of oospores was achieved with these methods,

germination was erratic and the rate of germination was usually low. In this study, about 70 % of oospores of *P. infestans* germinated in 20 days when oospores from a 20-day-old culture were treated with 0.25 % $KMnO_4$ solution for 15 min before incubation on modified S+L medium under light. The method also has the advantage of being free from interference by residual mycelial fragments, chlamydospores, and sporangia present in the oospore suspension.

Oospores of *Phytophthora* usually required a dormant period of about one month prior to germination (RIBEIRO 1978). Our result showed that with the method described in this study more than 25 % of oospores obtained from 4-day-old culture of *P. infestans* were able to germinate. However, even with similar treatment essentially no oospores from the 20-day-old culture of *P. parasitica* germinated (ANN and KO 1988). Different species of *Phytophthora* therefore appear to have different light requirements for oospore germination. Exposure to light during growth and oospore formation is not required for high germination of oospores of *P. infestans.* However, for obtaining a high germination rate of oospores of *P. parasitica* such treatment is essential (ANN and KO 1988).

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